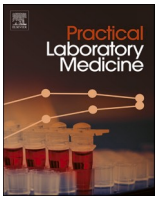
Practical Laboratory Medicine 39 (2024) e00361

Contents lists available at ScienceDirect

Practical Laboratory Medicine

journal homepage: www.elsevier.com/locate/plabm



Comparison of plasma aldosterone measured by chemiluminescence immunoassay and liquid chromatography-tandem mass spectrometry in screening test for primary aldosteronism

Wenzhan Chen [1], Fenghua Lai [1], Xiaoyu Huang, Shuang Yu, Nan Chen, Changliu Xu, Chenxue Wang, Shuhui Liang, Yanbing Li, Haipeng Xiao, Xiaopei Cao [*]

Department of Endocrinology, The First Affiliated Hospital, Sun Yat-Sen University, Guangzhou, China

ARTICLE INFO

Keywords:
Primary aldosteronism
Screening test
Chemiluminescence immunoassay
Liquid chromatography with tandem mass spectrometry

ABSTRACT

Background: Whether chemiluminescence immunoassay (CLIA) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) for plasma aldosterone concentration (PAC) measurement can be used interchangeably in primary aldosteronism (PA) screening is still controversial. The purpose of this study was to compare CLIA to LC-MS/MS for PAC measurement in PA screening.

Methods: All participants underwent aldosterone-to-renin ratio (ARR) testing. PA was diagnosed by captopril challenge test or saline infusion test. PAC in screening test was measured with CLIA and LC-MS/MS. Plasma direct renin concentration in screening and confirmatory test was measured with CLIA. The concordance between CLIA and LC-MS/MS for PAC measurement in PA screening was analyzed.

Results: Twenty-one healthy volunteers, 61 patients with essential hypertension (EH) and 43 PA patients were enrolled. Median PAC by CLIA was 84.7 % higher than that by LC-MS/MS in screening test ($P < 0.001$). A positive correlation of PAC was observed between the two assays (Pearson r coefficient 0.770, $P < 0.001$). When ARR was used in differentiating PA from EH, there was no difference in the area under the receiver operating characteristic curve between CLIA and LC-MS/MS for PAC measurement (0.968 *vs* 0.950, $P = 0.249$).

Conclusion: CLIA and LC-MS/MS for PAC measurement exhibited high and comparable efficacy in PA screening. CLIA is a reliable and feasible alternative in PA screening test.

1. Introduction

Primary aldosteronism (PA) is a common cause of secondary hypertension. It is characterized by inappropriate aldosterone production despite suppressed renin secretion and, commonly, hypertension and spontaneous hypokalemia [1]. The prevalence of PA was

* Corresponding author. 58 2nd Rd, 510080, Guangzhou, China.
E-mail address: caoxp@mail.sysu.edu.cn (X. Cao).
[1] These authors contributed equally to this work and should be considered as co-first authors.

https://doi.org/10.1016/j.plabm.2024.e00361
Received 10 October 2023; Received in revised form 14 January 2024; Accepted 18 January 2024
Available online 19 January 2024

estimated to be 5–10 % in all patients with hypertension [2–4], and it was reported to account for approximately 20 % of patients with resistant hypertension [5,6]. The latest data from the China Hypertension Survey indicated that the prevalence of hypertension among adult residents in China was 23.2 % (approximately 244.5 million) and the overall rate showed an increasing trend [7]. It was estimated that there might be at least 12 million patients with PA in China [8]. Compared with essential hypertension (EH), patients with PA are more likely to suffer from target organs damage and metabolic syndrome [9–13]. Some PA patients can be "cured" by surgery after a clear diagnosis, or the blood pressure can be well controlled by targeted drug therapy [14]. Therefore, routine screening for at-risk patients and early diagnosis of PA can greatly improve prognosis.

The diagnostic process of PA includes three steps: screening test, confirmatory test, and subtype classification [15]. Screening was considered indicative of PA based on plasma aldosterone concentration (PAC) and the aldosterone-to-renin ratio (ARR) [16]. ARR was recommended as the most reliable means of screening for PA by The Endocrine Society Clinical Practice Guideline [17]. At present, radioimmunoassay (RIA), chemiluminescence immunoassay (CLIA), and liquid chromatography with tandem mass spectrometry (LC-MS/MS) are the recommended methods for PA screening. The growing demand for screening of PA in hypertensive population necessitates the laboratories to switch into more automated assays, like plasma direct renin concentration (DRC) and PAC by CLIA. Previous study has shown that the automated CLIA assay for plasma DRC combined with LC-MS/MS for PAC measurement provided a rapid, reliable, and specific method for screening of PA [18]. Although LC-MS/MS was considered the "gold standard" for hormone detection and had the advantage of preventing non-specific reactions while avoiding interference from cross-reactions [19], the use of LC-MS/MS assay has largely been limited to reference laboratories and large academic centers. The challenges in the wide use of LC-MS/MS for clinical care are primarily a result of the manual nature of the assay, lack of integrated automation, and high capital expense [20]. Several studies have compared CLIA and LC-MS/MS for PAC measurement [21–24]. However, whether these two PAC measures can be used interchangeably is still controversial.

In the present study, we first evaluated the concordance between CLIA and LC-MS/MS for PAC measurement in healthy volunteers and patients at a high risk of PA, then compared the efficacy of these two methods for PA screening in at-risk patients.

2. Methods

2.1. Study population

Patients with suspected PA who were referred to the First Affiliated Hospital of Sun Yat-sen University from July 2021 to December 2021 were enrolled in the study. The criteria for suspected PA included: 1) persistent hypertension (above 150/100 mmHg on each of three measurements obtained on different days); 2) hypertension (>140/90 mmHg) resistant to three conventional antihypertensive drugs (including a diuretic) or controlled blood pressure on $\geq$ 4 antihypertensive drugs; 3) hypertension with spontaneous or diuretic-induced hypokalemia; 4) hypertension with adrenal incidentaloma; 5) hypertension with a family history of early onset hypertension or cerebrovascular accident at a young age (<40 years); 6) hypertension with a first-degree relatives of PA; or 7) hypertension with obstructive sleep apnea-hypopnea syndrome, according to the 2016 Endocrine Society's Clinical Practice Guideline [17]. Patients were excluded if: 1) age <18 years; 2) pregnancy; 3) treatment with steroids or oral contraceptives; 4) estimated glomerular filtration rate <30 mL/min/1.73 m^2; 5) renal artery stenosis; 6) other endocrine-related hypertensions (e.g., pheochromocytoma, Cushing's syndrome, pheochromocytoma and paraganglioma, hyperthyroidism). Twenty-one healthy volunteers (without any condition of suspected PA and any chronic disease) were also recruited. All included participants underwent physical examination and serum potassium/sodium measurement. Hypokalemia was defined as serum potassium <3.5 mmol/L.

This study was reviewed and approved by the Institutional Research Ethics Committee of the First Affiliated Hospital of Sun Yat-sen University (No. [2021]310). Informed written consent was obtained from each participant.

2.2. Screening test

Before screening test, antihypertensive medication was withheld or changed according to the guideline [17]. Treatment with diuretics among patients with hypertension was withheld for at least 4 weeks. Angiotensin-converting enzyme inhibitors, β-Blockers, and angiotensin-II receptor blockers were stopped for at least 2 weeks. Only the non-dihydropyridine calcium channel blocker, α-adrenergic blocker, and vasodilator were allowed for uncontrolled hypertension. Healthy volunteers and patients with suspected PA underwent screening test. For screening, samples for plasma DRC and PAC were collected in the morning after the subjects were out of bed for at least 2 h.

2.3. Diagnostic criteria

Considering the safety and convenience of diagnostic tests, we selected the captopril challenge test (CCT) as the first confirmatory test for all included patients with suspected PA. For patients who tested negative during CCT, if PA was strongly suspected based on young age, hypokalemia, or resistant hypertension, a second confirmatory test, the saline infusion test (SIT), was conducted. The diagnosis of PA was established if PAC was abnormally unsuppressed post-CCT (<30 %) or PAC of post-SIT was >10 ng/dL [17].

For the CCT, patients received 50-mg captopril orally at 7:00 to 8:00 a.m. after sitting or standing for at least 2 h. Blood samples were drawn for the measurement of DRC and PAC at baseline and 2 h after the challenge, with the patient remaining in an upright body position during this period.

For the SIT, patients stayed in the recumbent position for at least 1 h before and during the infusion of 2-L of 0.9 % saline over 4 h,

starting at 8:00 a.m. Blood samples were drawn at baseline and after 4 h for DRC and PAC determination. During the test, blood pressure and heart rate were closely monitored, and remained seated.

2.4. Sample collection

One week before blood sample collection, participants were asked to maintain their regular dietary intake and avoid coffee, strong tea the day before blood collection. Plasma samples were collected in the morning after participants were out of bed for at least 2 h and sitting for 5–15 min. Five milliliters of blood from a venipuncture were collected into tubes containing EDTA-K2. The plasma tubes were thoroughly mixed 8 to 10 times and centrifuged at 1200×*g* for 10 min at room temperature after being collected for 15–30 min.

2.5. CLIA method for DRC and PAC measurement

DRC in screening and confirmatory test was measured by automated CLIA (AutoLumo A2000, Autobio, Zhengzhou, China). The limit of detection for DRC was 0.05 ng/dL. The intra- and inter-assay coefficients of variation for DRC were <5 % and <15 %, respectively. PAC in screening test was measured by CLIA (AutoLumo A2000, Autobio, Zhengzhou, China). The limit of detection for PAC by CLIA was 0.5 ng/dL. The intra- and inter-assay coefficients of variation for PAC by CLIA were <6 % and <9 %, respectively.

2.6. LC-MS/MS method for PAC measurement

PAC in screening test and confirmatory test was measured by LC-MS/MS (ACQUITY UPLC I-Class/Xevo TQ-S IVD System). Plasma samples were extracted with dichloromethane/ether and thereafter evaporated and redissolved. After the separation on a reversed-phase column run was performed, a triple–quadrupole mass spectrometer and multiple reactions monitoring technique was used in the negative electrospray ionization mode. The limit of detection for PAC by LC-MS/MS was 2 ng/dL. The intra- and inter-assay coefficients of variation for PAC by LC-MS/MS were both <8 %.

2.7. Statistical analysis

For baseline characteristics, continuous variables with normal distributions and skewed distributions were described as mean ± standard deviation (SD) and median (interquartile range, IQR), respectively. The categorical variables were described as the number of cases (percentage). Comparisons between groups were performed using unpaired Student's *t* tests for continuous variables with normal distributions and homogeneity of variance, Mann-Whitney *U* tests for continuous variables with either skewed distributions or heterogeneity of variance, and χ^2 tests for categorical variables. Pearson correlation analysis was performed between CLIA and LC-MS/MS. Receiver operating characteristic (ROC) curves were generated to evaluate the performances of two methods among patients with suspected PA. The area under the ROC curve (AUC) values were calculated accordingly. The optimal cut-offs for diagnosis were determined based on the cut-off points with the maximum Youden's index. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated according to the confusion matrix. The 95 % confidence interval (CI) for each value was calculated by the exact Clopper-Pearson method [25]. A two-sided DeLong test was used to compare the AUC, sensitivity, specificity, PPV, and NPV of two methods [26]. All statistical analyses were performed using IBM SPSS Statistics 25.0 (IBM Corp., Armonk, NY, USA). *P* values were two-sided unless stated otherwise, and $P < 0.05$ was considered statistically significant.

Table 1
Clinical characteristics of the participants.

Characteristics	Healthy volunteer (N = 21)	EH (N = 61)	PA (N = 43)	*P* value *
Age (year), mean ± SD	48.2 ± 10.7	52.1 ± 10.5	46.9 ± 13.5	0.039
Sex, N (%)				0.097
Female	13 (61.9)	22 (36.1)	21 (48.8)	
Male	8 (38.1)	39 (63.9)	22 (51.2)	
Body mass index (kg/m^2), mean ± SD	22.8 ± 2.5	25.3 ± 3.4	24.7 ± 3.4	0.356
Duration of hypertension (month), median (IQR)	–	36.0 (12.0, 108)	88.0 (25.0, 180)	0.021
Systolic blood pressure (mmHg), mean ± SD	115.2 ± 11.3	150.5 ± 16.4	150.7 ± 24.3	0.956
Diastolic blood pressure (mmHg), mean ± SD	75.6 ± 8.7	96.3 ± 12.6	94.1 ± 11.8	0.068
Serum sodium (mmol/L), median (IQR)	140.0 (138.0, 142.0)	141.0 (139.0, 143.0)	143.0 (140.0, 145.0)	0.020
Serum potassium (mmol/L), median (IQR)	3.9 (3.7, 4.2)	3.9 (3.7, 4.1)	3.5 (3.1, 4.0)	<0.001
DRC in screening test (ng/dL),median (IQR)	2.6 (1.4, 3.1)	1.6 (1.1, 3.3)	0.3 (0.2, 0.6)	<0.001

**P* value for comparison of clinical characteristics between EH and PA groups.
EH, essential hypertension; IOR, interquartile range; PA, primary aldosteronism; SD, standard deviation.

3. Results

3.1. Basic characteristics of the study population

Twenty-one healthy volunteers, 61 patients with EH and 43 PA patients were enrolled. Baseline characteristics of the study population were summarized in Table 1. The age of healthy volunteers, EH patients, and PA patients was 48.2 ± 10.7, 52.1 ± 10.5, and 46.9 ± 13.5 years, respectively. Patients with PA were younger ($P = 0.039$) and had longer duration of hypertension (median month: 88.0 *vs* 36.0, $P = 0.021$) than patients with EH. Compared with EH patients, PA patients showed significantly higher serum sodium (143.0 [140.0, 145.0] mmol/L *vs* 141.0 [139.0, 143.0] mmol/L, $P = 0.020$), lower serum potassium (3.5 [3.1, 4.0] mmol/L *vs* 3.9 [3.7, 4.1] mmol/L, $P < 0.001$), and lower DRC (0.3 [0.2, 0.6] ng/dL *vs* 1.6 [1.1, 3.3] ng/dL, $P < 0.001$) in screening test. There were no significant differences in the variables of sex, body mass index, and blood pressure between EH and PA patients (all $P > 0.05$).

3.2. Comparison of PAC measured by CLIA and LC-MS/MS

In the screening test, comparison between the two PAC assays revealed the median PAC by CLIA was 84.7 % higher than PAC by LC-MS/MS among all participants (26.6 [18.8, 38.4] ng/dL *vs* 14.4 [8.0, 22.5] ng/dL, $P < 0.001$. Fig. 1). In the subgroup analyses, the median PAC by CLIA in healthy volunteers, EH patients, and PA patients were also higher than PAC by LC-MS/MS (18.6 [16.1, 27.2] ng/dL *vs* 13.7 [10.6, 24.1] ng/dL, 24.7 [18.3, 33.5] ng/dL *vs* 11.7 [7.7, 18.1] ng/dL, and 34.7 [26.6, 69.1] ng/dL *vs* 17.4 [12.4, 39.7] ng/dL, respectively, all $P < 0.05$).

3.3. Correlation between PAC by CLIA and LC-MS/MS

Among all participants, the Pearson *r* coefficient between PAC by CLIA and LC-MS/MS was 0.770 ($P < 0.001$, Fig. 2A). Similarly, in the subgroup of healthy volunteers (Fig. 2B), EH patients (Fig. 2C), and PA patients (Fig. 2D), positive correlations of PAC were observed between the two assays ($r = 0.842$, $r = 0.674$, and $r = 0.770$, all $P < 0.001$).

3.4. Performance of two ARR values for screening PA

ROC curves analyses were conducted among patients with suspected PA. According to the guideline, a PAC suppression percentage in the CCT <30 % or the PAC of post-SIT >10 ng/dL was recommended to confirm PA. Using this method, 61 EH patients and 43 PA patients were included in the ROC curves analysis. When ARR was used in differentiating PA from EH, there was no difference in the AUCs between CLIA and LC-MS/MS for PAC measurement (0.968 [95 % CI 0.919–0.992] *vs* 0.950 [95 % CI 0.889–0.983], $P = 0.249$, Fig. 3). The CLIA-based ARR ([ng/dL]/[ng/dL]) optimal cut-off of 30.0 and the LC-MS/MS-based ARR ([ng/dL]/[ng/dL]) optimal cut-off of 20.0 provided quite equal sensitivity (0.930 [95 % CI 0.809–0.985] *vs* 0.930 [95 % CI 0.809–0.985], specificity (0.918 [95 % CI 0.819–0.973] *vs* 0.896 [95 % CI 0.798–0.942], PPV (0.889 [95 % CI 0.775–0.949] *vs* 0.883 [95 % CI 0.761–0.930], and NPV (0.949 [95 % CI 0.862–0.982] *vs* 0.946 [95 % CI 0.855–0.981] for screening of PA (Table 2, all $P > 0.05$).

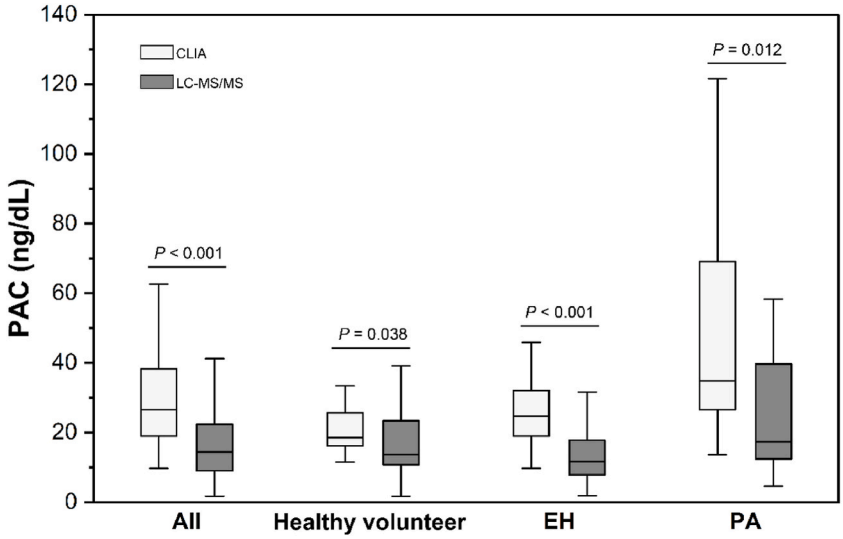


Fig. 1. Comparison of PAC measured by CLIA and LC-MS/MS.

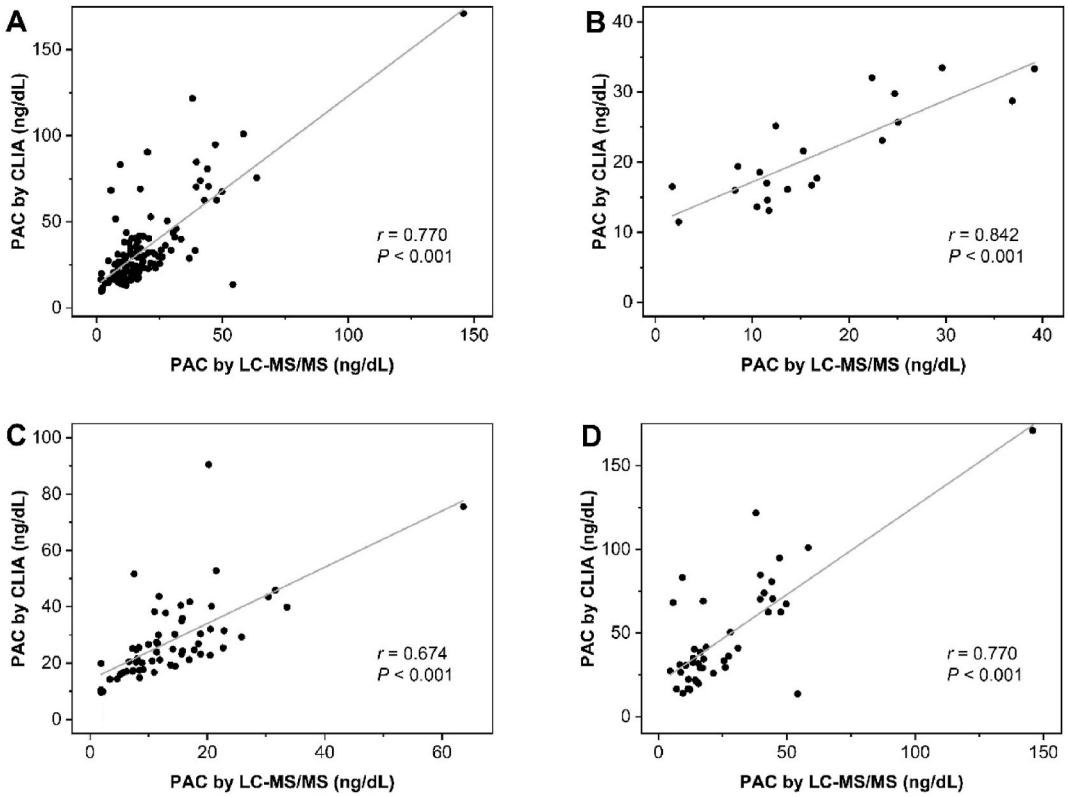


Fig. 2. Pearson correlations between PAC by CLIA and LC-MS/MS in all participants (A), healthy volunteer (B), EH patients (C), and PA patients (D).

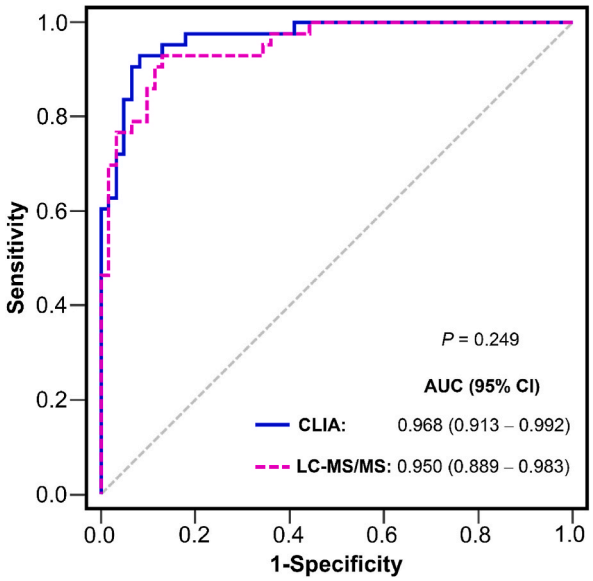


Fig. 3. Receiver operating characteristic curves analysis of ARR based on different PAC detection methods for PA screening.

4. Discussion

In the present study, the concordance between CLIA and LC-MS/MS for PAC measurement in PA screening was analyzed. We found that the median absolute value of PAC by CLIA was higher than that by LC-MS/MS. However, a positive correlation of PAC was observed between the two assays. Furthermore, The ARR by both pairs of the two methods showed a quite equal capacity to screen PA.

Table 2
Performance of ARR based on different PAC detection methods for PA screening.

	Optimal cut-off	Sensitivity (95 % CI)	Specificity (95 % CI)	PPV (95 % CI)	NPV (95 % CI)
$ARR_{PAC\ (CLIA,\ ng/dL)/DRC\ (CLIA,\ ng/dL)}$	30.0	0.930 (0.809–0.985)	0.918 (0.819–0.973)	0.889 (0.775–0.949)	0.949 (0.862–0.982)
$ARR_{PAC\ (LC\text{-}MS/MS,\ ng/dL)/DRC\ (CLIA,\ ng/dL)}$	20.0	0.930 (0.809–0.985)	0.896 (0.798–0.942)	0.883 (0.761–0.930)	0.946 (0.855–0.981)

ARR, aldosterone-to-renin ratio; CI, confidence interval; CLIA, chemiluminescence immunoassay; DRC, direct renin concentration; LC-MS/MS, liquid chromatography with tandem mass spectrometry; NPV, negative predictive value; PAC, plasma aldosterone concentration; PPV, positive predictive value.

Establishing the diagnosis of PA is often a multistep process. The diagnosis of PA starts with case detection, typically using ARR as the first-line screening test [27]. The ARR testing is easily measured in the outpatient setting. A normal or elevated PAC together with a low or suppressed renin is characteristic of PA and gives rise to an elevated ARR. The ARR threshold considered to be abnormal is crucially dependent on the assays used to measure aldosterone and renin. Conventionally, the ARR was calculated by measuring the PAC and plasma renin activity (PRA). However, more and more studies have found that the use of PAC and DRC instead of PRA to calculate ARR can effectively avoid the influence of angiotensinogen concentration, incubation conditions, pH value, and other factors on the measurement results [28–30]. A recent meta-analysis pooled 14 studies involving 2638 patients demonstrated that diagnostic efficacy of ARR calculated by DRC was higher than that calculated by PRA [31]. Therefore, DRC in screening test was used in our present study. We found that PA patients showed significantly lower plasma DRC than EH patients.

Accurate measurement of PAC is essential for screening and diagnosing PA. Among numerous assays to quantify PAC, RIA has been mostly used in routine clinical laboratories over the past few years. However, the procedure is complicated and there is a risk of radioactive hazards. In the past decades, there has been growing interest in quantifying PAC by using LC-MS/MS. Although LC-MS/MS has been reported to be more reliable than traditional RIA method [32–34], the promotion and development of this technology is hindered by its high cost and high technical threshold. LC-MS/MS assay involves multi-parameter optimization, requiring experienced technicians and a perfect quality control system [35]. In addition, there are no regulations and operating guidelines for LC-MS/MS analysis in China currently, thus limiting the application and development of this technology in clinical laboratories. CLIA have been rapidly developed for quantifying PAC owing to its advantages of time effectiveness, practicality, and the lack of radioactive contamination [36]. In the present study, we compared CLIA to LC-MS/MS for PAC measurement in PA screening and found that these two methods exhibited high and comparable efficacy. In view of the practicality and convenience of CLIA for PAC measurement, it may be a more feasible alternative in screening test.

Furthermore, the present study showed that PAC determined via CLIA was higher than that determined via LC-MS/MS analysis, which was consistent with previous findings [23,37,38]. One possible reason is that LC-MS/MS only detects aldosterone in plasma, while CLIA detects total aldosterone including aldosterone and aldosterone 3C-glucuronide [39]. Therefore, CLIA assay may be better to reveal pathological concentrations of aldosterone against which clinical decisions are based.

The findings of current study should be considered in the context of several limitations. First, the enrollment of PA patients was disproportionate compared with an actual prevalence of PA in general hypertensive cohorts. Second, only one CLIA platform was evaluated in the present study. It is important to note that the performance of CLIA assays for PAC measurement can vary between different kits/platforms. Finally, the generalizability of our results might be limited by the single-center design and small sample size. Therefore, further large multi-center studies with a large sample size and more CLIA platforms are needed to validate our findings.

5. Conclusion

In conclusion, the present study provided further information about the harmonization of PAC measurement by CLIA and LC-MS/MS. Consistency was observed between the two assays. CLIA and LC-MS/MS for PAC measurement exhibited high and comparable efficacy in PA screening. CLIA may be a more feasible alternative in screening test as it was much more practical and convenient.

CRediT authorship contribution statement

Wenzhan Chen: Writing – original draft, Methodology, Investigation, Data curation. **Fenghua Lai:** Writing – original draft, Methodology, Funding acquisition, Data curation. **Xiaoyu Huang:** Validation, Data curation. **Shuang Yu:** Validation, Data curation. **Nan Chen:** Formal analysis. **Changliu Xu:** Software. **Chenxue Wang:** Visualization. **Shuhui Liang:** Visualization. **Yanbing Li:** Supervision. **Haipeng Xiao:** Resources. **Xiaopei Cao:** Writing – review & editing, Project administration, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgment

This study was funded by China Postdoctoral Science Foundation (2022M723614) and the Research Fund of Medical Science and Technology of Guangdong Province (A2023093).

References

[1] K.W. Choy, P.J. Fuller, G. Russell, Q. Li, M. Leenaerts, J. Yang, Primary aldosteronism, BMJ 377 (2022) e65250, https://doi.org/10.1136/bmj-2021-065250.
[2] S. Monticone, J. Burrello, D. Tizzani, C. Bertello, A. Viola, F. Buffolo, L. Gabetti, G. Mengozzi, T.A. Williams, F. Rabbia, F. Veglio, P. Mulatero, Prevalence and clinical manifestations of primary aldosteronism encountered in primary care Practice, J. Am. Coll. Cardiol. 69 (14) (2017) 1811–1820, https://doi.org/10.1016/j.jacc.2017.01.052.
[3] G. Piaditis, A. Markou, L. Papanastasiou, I.I. Androulakis, G. Kaltsas, Progress in aldosteronism: a review of the prevalence of primary aldosteronism in pre-hypertension and hypertension, Eur. J. Endocrinol. 172 (5) (2015) R191–R203, https://doi.org/10.1530/EJE-14-0537.
[4] J. Widimský, J. Bruthans, P. Wohlfahrt, A. Krajčoviechová, P. Šulc, A. Linhart, J. Filipovský, V. Lánská, R. Cífkova, Primary aldosteronism in a general population sample. The Czech post-MONICA study, Blood Press 29 (3) (2020) 191–198, https://doi.org/10.1080/08037051.2020.1723406.
[5] J.M. Brown, M. Siddiqui, D.A. Calhoun, R.M. Carey, P.N. Hopkins, G.H. Williams, A. Vaidya, The unrecognized prevalence of primary aldosteronism: a cross-sectional study, Ann. Intern. Med. 173 (1) (2020) 10–20, https://doi.org/10.7326/M20-0065.
[6] B. Strauch, T. Zelinka, M. Hampf, R. Bernhardt, J.J. Widimsky, Prevalence of primary hyperaldosteronism in moderate to severe hypertension in the Central Europe region, J. Hum. Hypertens. 17 (5) (2003) 349–352, https://doi.org/10.1038/sj.jhh.1001554.
[7] Z. Wang, Z. Chen, L. Zhang, X. Wang, G. Hao, Z. Zhang, L. Shao, Y. Tian, Y. Dong, C. Zheng, J. Wang, M. Zhu, W.S. Weintraub, R. Gao, China hypertension Survey investigators, status of hypertension in China: results from the China hypertension Survey, Circulation 137 (22) (2018) 2344–2356, https://doi.org/10.1161/CIRCULATIONAHA.117.032380, 2012-2015.
[8] J. Lu, Y. Lu, X. Wang, X. Li, G.C. Linderman, C. Wu, X. Cheng, L. Mu, H. Zhang, J. Liu, M. Su, H. Zhao, E.S. Spatz, J.A. Spertus, F.A. Masoudi, H.M. Krumholz, L. Jiang, Prevalence, awareness, treatment, and control of hypertension in China: data from 1.7 million adults in a population-based screening study (China PEACE Million Persons Project), Lancet 390 (10112) (2017) 2549–2558, https://doi.org/10.1016/S0140-6736(17)32478-9.
[9] S. Monticone, F. D'Ascenzo, C. Moretti, T.A. Williams, F. Veglio, F. Gaita, P. Mulatero, Cardiovascular events and target organ damage in primary aldosteronism compared with essential hypertension: a systematic review and meta-analysis, Lancet Diabetes Endocrinol. 6 (1) (2018) 41–50, https://doi.org/10.1016/S2213-8587(17)30319-4.
[10] S. Monticone 1, E. Sconfienza, F. D'Ascenzo, F. Buffolo, F. Satoh, L.A. Sechi, F. Veglio, P. Mulatero, Renal damage in primary aldosteronism: a systematic review and meta-analysis, J. Hypertens. 38 (1) (2020) 3–12, https://doi.org/10.1097/HJH.0000000000002216.
[11] J. Hu, H. Shen, P. Huo, J. Yang, P.J. Fuller, K. Wang, Y. Yang, L. Ma, Q. Cheng, L. Gong, W. He, T. Luo, M. Mei, Y. Wang, Z. Du, R. Luo, J. Cai, Q. Li, Y. Song, S. Yang, Heightened cardiovascular risk in hypertension associated with renin-independent aldosteronism versus renin-dependent aldosteronism: a collaborative study, J. Am. Heart Assoc. 10 (24) (2021) e23082, https://doi.org/10.1161/JAHA.121.023082.
[12] C. Hung, S. Sung, C. Liao, C. Pan, C. Chang, Z. Chen, V. Wu, C. Chen, H. Cheng, Y. Lin, TAIPAI study group, aldosterone induces vascular damage, Hypertension 74 (3) (2019) 623–629, https://doi.org/10.1161/HYPERTENSIONAHA.118.12342.
[13] J. McCarthy, J. Yang, B. Clissold, M.J. Young, P.J. Fuller, T. Phan, Hypertension management in stroke prevention: time to consider primary aldosteronism, Stroke 52 (10) (2021) e626–e634, https://doi.org/10.1161/STROKEAHA.120.033990.
[14] K.S. Park, J.H. Kim, Y.S. Yang, A.R. Hong, D. Lee, M.K. Moon, S.H. Choi, C.S. Shin, S.W. Kim, S.Y. Kim, Outcomes analysis of surgical and medical treatments for patients with primary aldosteronism, Endocr. J. 64 (6) (2017) 623–632, https://doi.org/10.1507/endocrj.EJ16-0530.
[15] M. Reincke, I. Bancos, P. Mulatero, U.I. Scholl, M. Stowasser, T.A. Williams, Diagnosis and treatment of primary aldosteronism, Lancet Diabetes Endocrinol. 9 (12) (2021) 876–892, https://doi.org/10.1016/S2213-8587(21)00210-2.
[16] J. Funder, Primary aldosteronism, Trends Cardiovasc. Med. 32 (4) (2022) 228–233, https://doi.org/10.1016/j.tcm.2021.03.005.
[17] J.W. Funder, R.M. Carey, F. Mantero, M.H. Murad, M. Reincke, H. Shibata, M. Stowasser, W.F. Young, The management of primary aldosteronism: case detection, diagnosis, and treatment: an endocrine society clinical Practice guideline, J. Clin. Endocrinol. Metab. 101 (5) (2016) 1889–1916, https://doi.org/10.1210/jc.2015-4061.
[18] A. Juutilainen, K. Savolainen, J. Romppanen, U. Turpeinen, E. Hämäläinen, J. Kemppainen, L. Moilanen, K. Pulkki, Combination of LC-MS/MS aldosterone and automated direct renin in screening for primary aldosteronism, Clin. Chim. Acta 433 (2014) 209–215, https://doi.org/10.1016/j.cca.2014.03.015.
[19] Y. Yin, S. Yu, L. Qiu, X. Wang, D. Wang, C. Ma, K. Zhang, T. You, Establishment of a rapid and simple liquid chromatography tandem mass spectrometry method for measuring aldosterone in urine, J. Chromatogr., B: Anal. Technol. Biomed. Life Sci. 1113 (2019) 84–90, https://doi.org/10.1016/j.jchromb.2019.03.012.
[20] S.N. Thomas, D. French, P.J. Jannetto, B.A. Rappold, W.A. Clarke, Liquid chromatography-tandem mass spectrometry for clinical diagnostics, Nat Rev Methods Primers 2 (1) (2022) 96, https://doi.org/10.1038/s43586-022-00175-x.
[21] J.G. Van Der Gugten, J. Dubland, H. Liu, A. Wang, C. Joseph, D.T. Holmes, Determination of serum aldosterone by liquid chromatography and tandem mass spectrometry: a liquid-liquid extraction method for the ABSCIEX API-5000 mass spectrometry system, J. Clin. Pathol. 65 (5) (2012) 457–462, https://doi.org/10.1136/jclinpath-2011-200564.
[22] G. Eisenhofer, T. Dekkers, M. Peitzsch, A.S. Dietz, M. Bidlingmaier, M. Treitl, T.A. Williams, S.R. Bornstein, M. Haase, L.C. Rump, H.S. Willenberg, F. Beuschlein, J. Deinum, J.W.M. Lenders, M. Reincke, Mass spectrometry-based adrenal and peripheral venous steroid profiling for subtyping primary aldosteronism, Clin. Chem. 62 (3) (2016) 514–524, https://doi.org/10.1373/clinchem.2015.251199.
[23] E. Hinchliffe, S. Carter, L.J. Owen, B.G. Keevil, Quantitation of aldosterone in human plasma by ultra high performance liquid chromatography tandem mass spectrometry, J. Chromatogr., B: Anal. Technol. Biomed. Life Sci. 913–914 (2013) 19–23, https://doi.org/10.1016/j.jchromb.2012.11.013.
[24] Y. Yin, C. Ma, S. Yu, W. Liu, D. Wang, T. You, Q. Cheng, L. Qiu, Comparison of three different chemiluminescence assays and a rapid liquid chromatography tandem mass spectrometry method for measuring serum aldosterone, Clin. Chem. Lab. Med. 58 (1) (2019) 95–102, https://doi.org/10.1515/cclm-2019-0706.
[25] C.J. Clopper, E.S. Pearson, The use of confidence or fiducial limits illustrated in the case of binomial, Biometrika 4 (1934) 404–413.
[26] E.R. DeLong, D.M. DeLong, D.L. Clarke-Pearson, Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach, Biometrics 44 (3) (1988) 837–845.
[27] W.J. Young, Diagnosis and treatment of primary aldosteronism: practical clinical perspectives, J. Intern. Med. 285 (2) (2019) 126–148, https://doi.org/10.1111/joim.12831.
[28] D. Gruson, D. Maisin, P. Lison, D. Maiter, A. Persu, Two-site automated chemiluminescent assay for measurement of immunoreactive renin, Biomarkers 16 (7) (2011) 605–609, https://doi.org/10.3109/1354750X.2011.614015.
[29] T. Li, Y. Ma, Y. Zhang, Y. Liu, T. Fu, R. Zhang, K. Kang, Y. Yang, L. Wang, Y. Jiang, Y. Lu, Feasibility of screening primary aldosteronism by aldosterone-to-direct renin concentration ratio derived from chemiluminescent immunoassay measurement: diagnostic accuracy and cutoff value, Int. J. Hypertens. 2019 (2019) 2195796, https://doi.org/10.1155/2019/2195796.

[30] W. Lin, Y. Li, D. Chen, Z. Yao, H. Xu, Y. Chen, J. Xiao, P. Feng, W. Gan, High efficiency and problems of chemiluminescence assay-detected aldosterone-to-renin ratio in practical primary aldosteronism screening, Int. J. Hypertens. 2020 (2020) 3934212, https://doi.org/10.1155/2020/3934212.
[31] Z. Liu, X. Deng, L. Luo, S. Li, M. Li, Q. Deng, W. Zhong, Q. Luo, Diagnostic value of aldosterone to renin ratio calculated by plasma renin activity or plasma renin concentration in primary aldosteronism: a meta-analysis, Chin. Med. J. 135 (6) (2022) 639–647, https://doi.org/10.1097/CM9.0000000000001906.
[32] V.F. Fredline, P.J. Taylor, H.M. Dodds, A.G. Johnson, A reference method for the analysis of aldosterone in blood by high-performance liquid chromatography-atmospheric pressure chemical ionization-tandem mass spectrometry, Anal. Biochem. 252 (2) (1997) 308–313, https://doi.org/10.1006/abio.1997.2340.
[33] P.J. Taylor, D.P. Cooper, R.D. Gordon, M. Stowasser, Measurement of aldosterone in human plasma by semiautomated HPLC-tandem mass spectrometry, Clin. Chem. 55 (6) (2009) 1155–1162, https://doi.org/10.1373/clinchem.2008.116004.
[34] P.J. Taylor, S.P. van Rosendal, J.S. Coombes, R.D. Gordon, M. Stowasser, Simultaneous measurement of aldosterone and cortisol by high-performance liquid chromatography-tandem mass spectrometry: application to dehydration-rehydration studies, J. Chromatogr., B: Anal. Technol. Biomed. Life Sci. 878 (15–16) (2010) 1195–1198, https://doi.org/10.1016/j.jchromb.2010.03.033.
[35] A.H.B. Wu, D. French, Implementation of liquid chromatography/mass spectrometry into the clinical laboratory, Clin. Chim. Acta 420 (2013) 4–10, https://doi.org/10.1016/j.cca.2012.10.026.
[36] C. Schirpenbach, L. Seiler, C. Maser-Gluth, F. Beuschlein, M. Reincke, M. Bidlingmaier, Automated chemiluminescence-immunoassay for aldosterone during dynamic testing: comparison to radioimmunoassays with and without extraction steps, Clin. Chem. 52 (9) (2006) 1749–1755, https://doi.org/10.1373/clinchem.2006.068502.
[37] J.A. Ray, M.M. Kushnir, J. Palmer, S. Sadjadi, A.L. Rockwood, A.W. Meikle, Enhancement of specificity of aldosterone measurement in human serum and plasma using 2D-LC-MS/MS and comparison with commercial immunoassays, J. Chromatogr., B: Anal. Technol. Biomed. Life Sci. 970 (2014) 102–107, https://doi.org/10.1016/j.jchromb.2014.08.042.
[38] Y. Yin, C. Ma, S. Yu, W. Liu, D. Wang, T. You, Q. Cheng, L. Qiu, Comparison of three different chemiluminescence assays and a rapid liquid chromatography tandem mass spectrometry method for measuring serum aldosterone, Clin. Chem. Lab. Med. 58 (1) (2019) 95–102, https://doi.org/10.1515/cclm-2019-0706.
[39] F. Blocki, C. Zierold, G. Olson, J. Seeman, S. Cummings, F. Bonelli, In defense of aldosterone measurement by immunoassay: a broader perspective, Clin. Chem. Lab. Med. 55 (4) (2017) e87–e89, https://doi.org/10.1515/cclm-2016-0707.